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ANTIOXIDANT ACTIVITY AND CYTOTOXICITY OF POLYFLUORINATED 1, 4- NAPHTHOQUINONES WITH ALKYLATING FUNCTIONS IN THE QUINONE MOIETY

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ABSTRACT

Fluorinated derivatives of 1,4-naphthoquinones are highly potent inhibitors of Cdc25A and Cdc25B phosphatases and growth of tumor cells. Four derivatives of polyfluoro-1,4-naphthoquinone containing alkylating 2-chloroethylthio-group were synthesized for the first time and their cytotoxicity in human myeloma, human mammary adenocarcinoma, mouse and hamster fibroblasts cells as well as their mutagenic and antioxidant properties in a *Salmonella* tester strain were studied. All these compounds are effective antioxidants and protect bacterial cells from spontaneous and H_2O_2-induced mutagenesis. Taking into account these data together with the better cytotoxic effect against cancer cells as compared with normal mammalian cells, protecting of bacterial cells from spontaneous and H_2O_2-dependent mutagenesis, and low general toxicity towards different cells, one can propose these four compounds can be considered as useful potential antioxidants and inhibitors of tumor cells growth.

KEYWORDS: Alkylating Fluorinated Derivatives of 1, 4-Naphthoquinones, Antitumor Compounds

INTRODUCTION

Mammalian cell communication and growth is regulated by protein phosphorylation, which is the product of a dynamic balance between the enzymatic activity of protein kinases and phosphatases. The dual specificity protein phosphatase subfamily retains some of the structural attributes of PTPases but is unique in its ability to hydrolyze both phosphoserine/threonine as well as phosphotyrosine residues on the same protein substrate. Important members of the dual specificity phosphatase family are the Cdc25 phosphatases, which control cell cycle progression. Members of the Cdc25 family of phosphatases remove phosphates from cyclin-dependent kinases (Cdk) and thus activate cyclin-Cdk complexes, which control cell cycle progression [(1, 2)]. Cdc25A and Cdc25B are overexpressed in a number of tumors of various origins, frequently showing correlations with higher-grade or more aggressive tumors and poor prognosis [(3)]. The putative involvement of the Cdc25 phosphatases in tumorigenesis makes these cell cycle regulators potential targets for cancer therapy [(4, 5)].

Small molecule inhibitors have provided valuable tools to decode the role of kinases and phosphatases participating in specific cellular signaling pathways, because they are generally reversible, nonquantal and cell permeative. Several of numerous substances investigated were found to inhibit Cdc25 family enzymes [6, 7], and NSC 95397 (2, 3-bis [(2-hydroxyethyl)thio]-1,4-naphthoquinone) from the National Cancer Institute Library was shown to be the most potent Cdc25 inhibitor [8]. *P* - Naphthoquinones and 7-aminoquinoline-5,8-quinones are core structures for the synthesis of potential inhibitors of Cdc25 phosphatases [8, 9], including NSC 663284 [10]. Cpd5, or 2-(2-mercaptoethanol)-3-methyl-1, 4-naphthoquinone, has proven to be effective for inhibition of Cdc25 phosphatases [11].

Although most quinones have been reported to inhibit Cdc25 by sulfhydryl arylation at the quinone moiety, due to redox properties they can also generate toxic oxygen species [12], which may cause toxicity to normal tissues and thus reduce their therapeutic utility. One strategy for overcoming the intrinsic toxicity of quinones might be to use derivatives that are more stable in their reduced state and thus are less likely to initiate formation of radicals and indiscriminately damage cells. Interestingly, fluorinated derivatives of 1,4-naphthoquinones are less prone to form reactive oxygen species (ROS); among several substances, the most potent inhibitor of Cdc25A and Cdc25B phosphatases was fluorinated Cpd5 (F-Cpd5) [13–15]. F-Cpd5 was calculated to have much higher reduction potential compared with Cpd5 and predicted not to generate ROS [13]. F-Cpd5 is three times more potent than Cpd5 in inhibiting Hep3B cell growth [13–15] and inhibits mitogen-induced DNA synthesis in normal rat hepatocytes 12-fold less than in Hep3B cells [14].

The rationale to study inhibitors of Cdc25 phosphatases is looking for better inhibitors of tumor cells growth having no toxic, mutagenic or carcinogenic properties. Since all naphthoquinones inhibit Cdc25 phosphatases [8–15], it is reasonable to suggest that some new polyfluorinated naphthoquinones will also inhibit these enzymes to some extent. As only the data concerning toxic, mutagenic or carcinogenic properties could allow to reliably judge possible applications of new compounds as antitumor drugs, a measurement of their affinity for Cdc25 phosphatases seems to be of secondary importance. Taking this into account, we have recently synthesized several new polyfluorinated *n*-alkylamino-, phenylamino- and alkylthioderivatives of 1,4-naphthoquinone and investigated their mutagenic and antioxidant properties using special type of *Salmonella* cells, as well as their cytotoxicity in human and mouse tumor cells, and primary mouse fibroblast cells [16–18]. Interestingly, for all these compounds the efficiency to suppress the growth of cancer cells at significantly lower concentrations than normal cells and antioxidant and mutagenic properties in the bacterial system significantly depend upon structure of the functional groups, only 3–4 of them possessing a highly promising combination of these properties [16–18]. Thus, this approach seems more rational for searching new inhibitors of Cdc25 phosphatases and tumor cell growth exerting no side effects.

In this work we have synthesized for the first time four alkylating fluorinated derivatives of 1,4-naphthoquinone which are capable not only interact reversible but in principle to modify target enzymes. Relative cytotoxicity of these compounds against cancer cells and ability to protect bacterial cells from spontaneous and H_2O_2-induced mutagenesis were compared.

MATERIALS AND METHODS

Chemistry

Commercially supplied 2-chloroethanol, Et_3N, acetonitrile and chloroform were purified by distillation. 2-Chloroethanethiol was prepared accordingly to reported method [19]. Quinones **5** and **6** were prepared by methods [18, 20], respectively. ^{19}F and ^{1}H spectra were recorded on a Bruker AV-300 NMR spectrometer at 282 MHz (^{19}F) or 300.13 MHz

(^{1}H) and chemical shifts were referenced to C_6F_6 and $CHCl_3$, respectively. The melting points were determined on a FP 900 Thermosystem microscope melting point apparatus (Mettler-Toledo International Inc., Zürich, Switzerland).

Procedures for the Synthesis of Quinones 1–4

2-(2-Chloroethylsulfanyl)-5, 6, 7, 8-tetrafluoro-3-methoxy-1,4-naphthoquinone **1.** A mixture of quinone **6 (**0.05 g, 0.18 mmol), 2-chloroethanethiol (0.07 g, 0.72 mmol) and CH_3CN (2 ml) was stirred under argon at room temperature for 8 days**.** Water (5 mL) was added, the product was extracted with CH_2Cl_2 and isolated by the subsequent TLC separation (TLC aluminium sheets «MERCK», chloroform) to yield quinone **1** (0.05 g, 80%), orange crystals, mp 90.5–91.5 ^{o}C. ^{19}F NMR: *δ*/ppm 24.4, 24.2 (AB-system, J_{AB} ~13 Hz, each component is ddd, $J_{5,6}$, $J_{8,7}$ ~19 Hz, $J_{5,7}$, $J_{8,6}$ ~11 Hz, F^5, F^8), 18.0 (ddd, $J_{6,5}$, $J_{6,7}$ ~19 Hz, $J_{6,8}$ ~11 Hz, F^6 or F^7), 17.2 (ddd, $J_{7,6}$, $J_{7,8}$ ~19 Hz, $J_{7,5}$ 10–12 Hz, F^7 or F^6). ^{1}H NMR: *δ*/ppm 4.20 (s, 3H, CH_3), 3.70, 3.44 (AB-system, J_{AB} = 7.6 Hz, each component is ddd, J = 0.7, 6.2, 6.8 Hz, 4H, $2CH_2$). HRMS (M^+, $C_{13}H_7F_4ClO_3S$) calcd 353.9735, found 353.9747.

2, 3-Bis-(2-chloroethylsulfanyl)-5, 6, 7, 8-tetrafluoro-1, 4-naphthoquinone **2.** A mixture of quinone **5** (0.05 g, 0.18 mmol), 2-chloroethanethiol (0.05 g, 0.54 mmol), Et_3N (0.04 g, 0.36 mmol) and chloroform (~10 mL) was stirred under argon at room temperature for 1 h**.** After water (~10 mL) was added, the organic layer was separated; the solvent was distilled off in vacuo. The remainder was dissolved in CH_2Cl_2 (5 mL) and purified by passing through a column with silica gel. The eluent was evaporated till ~1 mL, and precipitated by hexane was quinone **2** (0.06 g, 71%), yellow crystals, mp 114–115 ^{o}C. ^{19}F NMR: *δ*/ppm 24.6 (m, F^5, F^8), 17.6 (m, F^6, F^7). ^{1}H NMR: *δ*/ppm 3.76 (t, 4H, J = 6.8 Hz, CH_2), 3.55 (t, 4H, J = 6.8 Hz, CH_2). Elemental Anal. Calcd for $C_{14}H_8Cl_2F_4O_2S_2$: C 40.11; H 1.92; S 15.30. Found: C 40.13; H 1.91; S 15.23.

(2, 3-Bis-(2-chloroethoxy)-5,6,7,8-tetrafluoro-1,4-naphthoquinone **3.** A mixture of quinone **5** (0.10 g, 0.38 mmol), 2-chloroethanol (0.06 g, 0.75 mmol), Et_3N (0.08 g, 0.75 mmol) and CH_3CN (2 mL) was stirred at room temperature for 5 h. After water (~8 mL) was added, the precipitate was centrifuged off, washed with water (2×4 mL), dried in vacuo (0.03 mmHg) and crystallized from hexane with few drops of CH_2Cl_2 to yield **3** (0.106 g, 73%), yellow crystals, mp 124–125 ^{o}C. ^{19}F NMR: *δ*/ppm 24.3 (m, F^5, F^8), 17.8 (m, F^6, F^7). ^{1}H NMR: *δ*/ppm 4.63 (t, 4H, J = 5.5 Hz, CH_2), 3.86 (t, 4H, J = 5.5 Hz, CH_2). Elemental Anal. Calcd for $C_{12}H_6F_4O_4$: C, 43.44; H, 2.08. Found: C, 43.94; H, 2.23.

2-(2-Chloroethoxy)-3,5,6,7,8-pentafluoro-1,4-naphthoquinone 4**.** A mixture of quinone **6** (0.10 g, 0.38 mmol), 2-chloroethanol (0.03 g, 0.38 mmol), Et_3N (0.04 g, 0.38 mmol) and CH_3CN (2 mL) was stirred at room temperature for 30 min. After water (~10 mL) was added, the precipitate was centrifuged off, washed with water (2×2 mL), dissolved in CH_2Cl_2 (0.5 mL) and precipitated by hexane to yield **4** (0.096 g, 78%), yellow crystals, mp 168.5–169.5 ^{o}C. ^{19}F NMR: *δ*/ppm 26.0 (ddd, F^8 or F^5, $J_{8,5}$, $J_{8,6}$ 11–12 Hz, $J_{8,7}$ = 19.5 Hz), 25.7 (ddd, F^5 or F^8, $J_{5,6}$ = 19.5 Hz, $J_{5,7}$, $J_{5,8}$ 11–12 Hz), 22.5 (m, F^3), 19.4 (dddd, F^6, $J_{6,5}$, $J_{6,7}$ ~19.5 Hz, $J_{6,8}$ = 12.0 Hz, $J_{6,3}$ = 3.9 Hz), 18.9 (ddd, F^7, $J_{7,5}$ = 11.5 Hz, $J_{7,6}$, $J_{7,8}$ ~19.5 Hz). ^{1}H NMR: *δ*/ppm 3.82 (2H, t, J = 5.5, CH_2), 4.68 (2H, td, J = 5.5, $J_{H\text{-}F}$ 2.1, CH_2). HRMS ([M - Cl^-, - H]$^+$, $C_{12}H_3F_5O_3$) calcd 289.9997, found 290.0005. Elemental Anal. Calcd for $C_{12}H_3F_5O_3$: C, 44.13; H, 1.23. Found: C, 44.99; H, 1.31.

Determination of Mutagenisity of Compounds

In the Ames test, the histidine-dependent strain of *S. typhimurium* TA102 was used, which carries a mutation at the histidine operon [(21)]. The mutagenic activity of the samples was analyzed by the standard method without metabolic activation [(21)]. A liquid culture of TA102 was obtained by 16-h growth of cells from a frozen stock at 37°C in LB medium with penicillin. Then cells were plated on minimal glucose agar, antibiotics and histidine at the density sufficient to obtain

isolated colonies. A separate bacterial colony was inoculated into LB medium (5 mL) containing ampicillin (50 □g/mL) and tetracycline (2 □g/mL), and grown with shaking (130 rpm) for 15 h at 37°C.

The Ames test was carried out using the double-layer method as described in [21]. The overnight culture of bacteria (100 □l) containing one of the tested compounds in different concentrations and, if required, 3 mM H_2O_2, were mixed at 42°C with 2 mL of liquid 0.6% top agar. The mixture was poured onto plates with a minimal medium containing 0.2% glucose and 3% agar, taking care to distribute the mixture uniformly on the surface of the solid agar. The plates were incubated for 48 h at 37°C, and the revertants were counted. The cells incubated with H_2O_2 in the absence of compounds analyzed were used as positive controls, and the cells grown in the absence of H_2O_2 and antioxidants served as negative controls for mutation induction. The results are expressed as mean ± S.E. of at least 3 independent experiments.

Cytotoxicity Assays

Tumor cell lines from human myeloma RPMI 8226, human mammary adenocarcinoma MCF-7, mouse (LMTK) and hamster (Ag-17-1) fibroblast cell line (PMF) (~2000 cells per well) were incubated for 24 h at 37ºC in IMDM or RPMI 1640 medium (5% CO_2) and then were treated with compounds **1**–**4**. After 72 h of cell incubation, the relative amount of live cells was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (a standard colorimetric MTT-test [22]) and the drug concentration that caused 50% cell growth inhibition (IC_{50}) was determined. The results are expressed as mean ± S.E. of at least 3 independent experiments.

RESULTS

Chemistry

Alkylating compounds **1**–**4** were prepared via the reactions of 2-methoxy-3, 5, 6, 7, 8-pentafluoro-1, 4-naphthoquinone (**5**) with 2-chloroethanethiole (for **1**) and hexafluoro-1, 4-naphthoquinone (**6**) with 2-chlotoethanethiol (for **2**) or 2-chloroethanol (for **3** and **4**) (Scheme 1).

Quinone **5** reacted with $ClCH_2CH_2SH$ in CH_3CN at ~20°C to afford **1** in 80% isolated yield. Quinone **6** reacted easily with one equivalent of $ClCH_2CH_2OH$ in the presence of Et_3N in CH_3CN at ~20°C to afford **4** in 78% isolated yield. In the analogous reaction with two equivalents of $ClCH_2CH_2OH$ quinone **3** was obtained in 73% isolated yield. The interaction of **6** with one equivalent of $ClCH_2CH_2SH$ in the presence of Et_3N in $CHCl_3$ at ambient temperature gave (the ^{19}F NMR data) a mixture of comparable amounts of the starting quinone **6**, obviously 2-(2-chloroethyl) sulfanyl-3,5,6,7,8-pentafluoro-1,4-naphthoquinone (**7**) and quinone **2**. This result indicates at least not lesser reactivity of the mono-sulfanodefluorination product **7** compared with **6** for a further nucleophilic substitution, thus making difficult obtaining the individual **7**. The interaction of **6** with two equivalents of $ClCH_2CH_2SH$ under above conditions gave quinone **2** in 71% isolated yield.

Compounds **1**–**4** have been characterized by ^1H and ^{19}F NMR, elemental analysis or high resolution mass-spectroscopy. Their ^{19}F NMR spectra show two signals at δ 24–26 and 17–19.5 ppm belonging to the atoms F^5, F^8 and F^6, F^7, respectively, the δ values and spin coupling patterns being close to those of quinones **5** (F^5, F^8 δ 25.3; 25.8; F^6, F^7 δ 19.2, 18.4 ppm) and **6** (F^5, F^8 δ 25.5; F^6, F^7 δ 18.6 ppm). In the ^{19}F NMR spectrum of quinone **4**, F^3 exhibits a multiplet at δ 22.5 ppm, which is close to F^3 of quinone **6** (δ 22.1 ppm) but down-field shifted compared to F^3 of **5** (δ 17.5 ppm). The difference between the $\delta(F^3)$ values of **5** and **6** is reasonably explained by a stronger electron-donating effect of the MeO-group compared with fluorine thus increasing the F^3 shielding in **5** compared with **6**. Probably, the more bulky

$ClCH_2CH_2O$-group in **4** is out-of-plane rotated that somewhat suppresses its electron-donating effect compared with the MeO-group and returns the F^3 resonance to low field. The characteristics of the ^1H NMR spectra of quinones **1**–**4** are consistent with the proposed structures.

Biological Studies

At the first step of evaluation of biological properties of compounds containing alkylating groups **1–4**, we have analyzed their ability to inhibit the growth of two mammalian cell lines; tumor cell lines from human myeloma (RPMI 8226) and human mammary adenocarcinoma (MCF-7), as well as not cancer mouse (LMTK) and hamster (Ag17-1) fibroblasts cells.

Figure 1 shows representative data for compounds **1-4** in the case of RPMI cells. The results obtained for compounds **1**–**4** with all types of cells are summarized in Table 1. It can be seen that compound **2** and **3** demonstrated the best inhibition of RPMI cancer cells (1.2–1.3 □M), but suppressed the growth of MCF-7 cells at concentrations 2.1–2. 6-fold higher (2.7–5.1 □M) (Table 1). Compounds **4** inhibited the growth of cells of both cancer cell lines at nearly the same concentrations (2.0–2.2 μM), while derivative **1** had IC_{50} (2.0 □M) for RPMI cells 2.6-fold lower than that for MCF-7 cells (5.1 □M). (Table 1).

Antitumor drugs may be considered potentially more useful when they are better suppressors of tumor than normal mammalian cells. Therefore, we have compared the effects of these compounds on cancer cell lines and not cancer mouse (LMTK) and hamster (Ag-17-1) fibroblasts. Interestingly, all four compounds suppressed the growth of normal cells at higher concentrations than was observed for tumor cells. The ratios of concentrations inhibiting the growth of the two types of normal and two types of tumor cells were: 1.3–9.1 (compound **1**), 2.2-11.3 (**2**), 2.0–11.1 (**3**), 2.8–9.5 (**4**). Thus, the best difference in inhibition of tumor and normal cells was observed for compounds **2**, **3**, and **4**.

Recently we have compared the growth-inhibiting properties of F-Cpd5 with those for nineteen new polyfluoro-1,4-naphthoquinone derivatives [16–18]. Fifteen of them exhibited the growing of tumor cells in (2–72)-fold lower concentration than that for F-Cpd5 (IC_{50} = 14.8±0.9 μM for RPMI and 173.0±21.0 μM for MCF cells) [16–18].

The structures of **1**–**4** resemble that of F-Cpd5 and above mentioned quinones (I–VII), therefore one could expect similar effects of all these compounds on the Cdc25 phosphatases and growth of tumor cells. At the same time, alkylating compounds **1–4** in principle can irreversibly modify these phosphatases decreasing the effect on the growth of tumor cells.

For RPMI cancer cells, F-Cpd5 revealed IC_{50} 6.2–12.3-fold higher than IC_{50} of compounds **1–4,** while all **1–4** inhibited the growth of MCF cells (11–28)-fold better than F-Cpd5 (Table 1). The data suggest a common mechanism of cancer cell growth inhibition by F-Cpd5 [11–15], polyfluoro-1, 4-naphthoquinone derivatives I–XV, and compounds **1**–**4**.

It is known that some compounds interacting with many cell targets at the same time may be polyfunctional and possess cytoprotective properties, or, on the contrary, may be mutagenic or carcinogenic. Obviously, drugs are more successful when they are not mutagenic at least at the therapeutic concentrations. The *Salmonella typhimurium* TA102 strain is often used both for evaluation of mutagenicity of different compounds and for detection of antioxidant properties, as judged from suppression of spontaneous mutagenesis in this strain and from a decrease in mutagenicity of oxidants, usually H_2O_2 [21]. The mutagenic activity of compounds **1**–**4** was estimated in the Ames test [21] using *S. typhimurium* TA102 as reported by Kemeleva *et al.* [23]. The mutation induction in the Ames assay is estimated by calculating the

frequency of reversion from histidine auxotrophy to prototrophy in response to the substance under testing [21, 23].

Figure 2 shows the representative data for quinones **1** and **4**. They displayed higher potency (IC_{50} values 0.3–0.35 □M) in suppression of the spontaneous appearance of mutants than **2** and **3** (0.65–0.8□□M) (Table 2). Some antioxidant compounds are known to efficiently decrease the mutagenic effect of H_2O_2 [21, 23]. In the Ames test, H_2O_2 was added to TA102 cells at the optimal concentration, 3 mM [21], and the test compound concentrations varied (Figure 2). At low concentrations (0.23–0.33 □M) quinones **2** and **3** efficiently suppressed the H_2O_2-dependent formation of mutants from 130 to 100% of revertants (the number of revertants observed in controls without H_2O_2 was taken for 100%), but **1** and **4** did it at lower concentrations (0.11–0.045 □M) (Table 2). The efficiency of cell protection from effect of H_2O_2 decreased in the sequence 4 > 1 > 3 > 2 (Table 2).

The ratio of the concentrations corresponding to a decrease in mutant appearance from 130 to 100% and from 100 to 50% in the presence of H_2O_2 depended significantly on the compound: 5.0- (**1**), 2.6- (**2**), 3.1- (**3**), and 5.8-fold (**4**). These data indicate that quinones **1**–**4** are not mutagenic themselves and decrease the level of spontaneous mutagenesis as well as the mutagenic effect of H_2O_2.

Interestingly, in the absence of H_2O_2 the potency of quinone **2** in suppressing spontaneous mutagenesis (IC_{50} = 0.65 □□) was slightly lower than in decreasing H_2O_2-dependent mutagenesis (IC_{50} = 0.85 □□), while other compounds showed somewhat better IC_{50} values in the presence than in the absence of H_2O_2 (Table 2). Since **1**–**4** did not enhance the spontaneous mutagenesis and effectively suppressed the mutagenic effect of H_2O_2, they can be considered efficient antioxidants. It should be mentioned, that in comparison with many previously studied compounds [16–18], first synthesized quinones **1-4** demonstrate oxidative stress suppressing at lower concentrations. In addition, the 50% decrease in spontaneous and H_2O_2-dependent mutagenesis of bacterial cells for all four compounds was observed at comparable or lower concentrations (0.26–0.85 □□) than those having an effect in tumor cells (1.2–5.1 □□□□□Tables 1 and 2)

Our data suggest that compounds **1**–**4** demonstrate a similar or more pronounced decrease in tumor cells growth in comparison with control F-Cpd5, a potent inhibitor of Cdc25 phosphatases [13–15]. It is possible that all these compounds including F-Cpd5 play a double role, acting both as inhibitors of cell phosphatases and as antioxidants. Thus, alkylating fluorinated derivatives of 1,4-naphthoquinone inactive in generating ROS and may be more promising inhibitors of Cdc25 as compared to 1,4-naphthoquinone [13–15]. In addition, effects of alkylating derivatives may be increased after longer time of cells incubation, since they can irreversibly modify the phosphatases.

CONCLUSIONS

Our data indicate that quinones **1** and **4** are the most promising as effective antioxidants, while **2, 3**, and **4** demonstrate better cytotoxic effect against cancer cells. At the same time all four compounds show comparable properties in protection of cells from oxidative stress and in the inhibition of tumor cells. Taken together, our data suggest that quinones **1**–**4** may be considered as potentially useful inhibitors of growth of tumor cells.

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APPENDICES

LEGENDS TO FIGURES

Scheme 1: Synthesis of compounds **1-4**.

Figure 1: Effects of fluorinated compounds 1-4 (relative activity, RA) on the growth of MCF-7 cells. The average error in three experiments for any compound concentration did not exceed 5–15%.

Figure 2: Analysis of the mutagenic and antioxidant activity of compounds **2** (A) and **4** (B) by a standard Ames test using the *S. typhimurium* strain TA102 in the absence (■) and in the presence (□) of 3 mM H_2O_2 (A and B). The number of revertants in the absence of H_2O_2 was taken for 100%. The average error in three experiments for any compound concentration did not exceed 5–15%.

Table 1: Cytotoxicity (IC_{50}) of the Alkylating Fluorinated Derivatives of 1, 4-Naphthoquinone

No	Compound	IC_{50} (Mm) For Different Compounds*				
		RPMI (1)	MCF-7 (2)	LMTK (3)	Ag17-1 (4)	Diapason of Ratios of (3)-(4) and (1)-(2)
1	2-(2-chloroethylthio)-5,6,7,8-tetrafluoro-3-methoxy-1,4-naphthoquinone	2.0±0.2	5.1±0.5	6.6±0.6	18.1±1.6	1.3-9.1
2	2,3-bis(2-chloroethylthio)-5,6,7,8-tetrafluoro-1,4-naphthoquinone	1.2±0.1	2.9±0,3	6.3±0.6	13.5±1.2	2.2-11.3
3	(2,3-bis (2-chloroethoxy) -5,6,7,8-tetrafluoro-1,4-naphthoquinone	1.3±0.1	2.7±0,3	5.5±0.5	14.4±1.3	2.0-11.1
4	2-(2-chloroethoxy) 3,5,6,7,8-pentafluoro-1,4-naphthoquinone	2.4±0.2	2.0±0.2	5.6±0.5	22.9±1.9	2.8-9.5
Control	F-Cpd5:5,6,7,8-tetrafluoro-2-(2-mercaptoethanol)-3-methyl-[1,4]naphtoquinone	14.8±0.9	54.9 ± 6.7	No inhibit.at 173 □M	n.d.[§]	> 10

*Mean ± S.D. from three independent experiments.

[§]n.d., not determined.

Table 2: IC_{50} Values Characterizing Suppression of Spontaneous and H_2O_2-Induced Mutagenesis by Alkylating Polyfluorinated Derivatives of 1, 4-Naphthoquinone

Number	Compound	IC_{50}, □M*		
		Suppression of Spontaneous Mutagenesis (From 100 To 50%)	Suppression of H_2O_2-Induced and Spontaneous Mutagenesis	
			From 130 to 100%	From 100 to 50%
1	2-(2-chloroethylthio)-5,6,7,8-tetrafluoro-3-methoxy-1,4-naphthoquinone	0.35±0.04	0.11±0.01	0.55±0.05
2	2,3-bis(2-chloroethylthio)-5,6,7,8-tetrafluoro-1,4-naphthoquinone	0.65±0.05	0.33±0.03	0.85±0.07
3	(2,3-bis (2-chloroethoxy)-5,6,7,8-tetrafluoro-1,4-naphthoquinone	0.8±0.1	0.23±0.04	0.71±0.07
4	2-(2-chloroethoxy)3,5,6,7,8-pentafluoro-1,4-naphthoquinone	0.3±0.03	0.045±0.005	0.26±0.03
Control	F-Cpd5:5,6,7,8-tetrafluoro-2-(2-mercaptoethanol)-3-methyl-[1,4]naphtoquinone	0.81± 0.09	n.d.[§]	0.44± 0.05

*Mean ± S.D. from three independent experiments.

[§]n.d., not determined.